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THE REACTION OF ASTEROIDS ASTERIAS AMURENSIS AND DISTOLASTERIAS NIPON TO HOMOGENATES AND CHEMICAL SUBSTANCES FROM FAR EASTERN STARFISHES

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The reaction of asteroids Asterias amurensis and Distolasterias /40*
nipon to homogenates of tissues and substances extracted from five species of starfish was studied. Only "frightening" and zero reactions were observed in experiments. Both species reacted strongly and species-nonspecifically to homogenates of tissues of the pyloric ceca of starfishes, which is due to the lysing protease enzymes which they contain. The asteroids did not react (with one exception) to substances extracted from conspecific individuals. The asteroids react to the high-molecular protein fraction with m.m. >2000 and the protein fraction which is associated with inorganic substances. It is assumed that high-molecular proteins underlie the effect of the "frightening" substances in starfishes.

Chemical communication is one of the most important mechanisms /41
regulating the intra- and interspecific relations of aquatic organisms, including the widely distributed and numerous asteroids. The interrelations

*The numbers in the right-hand margin indicate the corresponding pages in the original. - Transl.

of starfishes is of both scientific and great practical importance, since their study can lead to methods of controlling the behavior of these predators which do considerable damage to plantations of cultivated molluscs.

There are a number of studies (survey: Sloan, 1980; Sloan, Northway, 1982) of the interactions (including chemical ones) of asteroids with individuals of the same and other species. However, the nature of the substances responsible for chemical communication in these animals remains unclarified.

In this paper, we have made an attempt to explain how the two most economically important starfish species of the sublittoral of the Sea of Japan - Asterias amurensis and Distolasterias nipon - react to homogenates of tissues and chemical substances extracted from the same and some other numerous starfish species.

Materials and procedure

The work was done at the aquarium of the "Vityaz'" experimental base of the Pacific Oceanological Institute of the Far Eastern Scientific Center of the USSR Academy of Sciences[1] in May-August 1982. The test objects were asteroids Asterias amurensis and Distolasterias nipon with a large radius of 8-12 cm. The active agents used in the experiments were isolated from five species of starfish - A. amurensis, D. nipon, Lysastrosoma anthosticta, Patiria pectinifera and Crossaster papposus. All the starfishes, except for C. papposus, which was taken in a drag net at Onekotan I. (Kurile Islands), were taken by divers in Vityaz' Bay in the Sea of Japan at depths of 5-30 m. The animals were kept in 1000L basins with aerated running water.

A device (Fig. 1, insert II) similar in design to that of Castilla

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[1]The authors are deeply obliged to V.P. Naidenko, the Aquarium Director, for permission to do the work.

and Crisp (1973) with water supply and drainage similar to that of Pratt (1974), was devised for the experiments. The device consists of a 150X50X15cm chute of polyvinyl chloride plastic 1 cm thick. The chute is divided by a longitudinal partition into two compartments, experimental and control. Grids were installed at a distance of 10 cm from both end walls to keep out debris and to prevent the starfishes from climbing into the intakes.

Water was fed into the device at the end faces from the aquarium pipes at the same rate in both compartments; the drain was situated in the middle of the chute. The average flow rate of water in each compartment was 2L/min (1 L/min on each side). The test substances were fed in in the form of a solution on one side of the experimental compartment through a separatory flask. Asteroids were placed in the compartment between the intakes and the grid in order to study their effect on the experimental species of animals.

During the experiment, the test asteroid was placed on the starting line in the middle of the compartment so that the forward ray (determined by the direction of movement of the asteroid immediately before the experiment) was directed perpendicularly to the possible direction of movement. If the asteroid had not moved in 5 min, or had moved along the starting line, a zero reaction was considered to have occurred; it it moved towards the stream of water with substance, a positive, and if in the opposite direction, a negative reaction was considered to have occurred.

In all, 145 experiments were done, in each of which 30 asteroid specimens were used. The experiment included 30 samples, each of which was used on two animals, the test and the control. The reliability of the total negative reaction in the experiment was determined by the φ method for sample indicators of qualitative characters (Plokhinskii,

1970). The proportion of negative reactions in the total number of reactions in the test and the control was compared. The estimate was done at a 95% confidence level.

Whole asteroids, homogenates, cold extracts, extracts and chemical substances from their tissues were used in the experiments (Fig. 2). The homogenates were obtained by grinding 20 g of animal tissue in a mortar with fine quartz sand with subsequent elution with 300 mL of water. The cold extracts from the body walls were prepared /42 by keeping 100 g of finely chopped tissues in 300 mL of fresh water for 24 h. The exudate was obtained by placing living asteroids in a vessel with fresh water; the water was decanted after 24 h and evaporated dry.

To prepare a total extract, the preliminary crushed animals were heated in ethyl alcohol in a flask with a reflux condenser; 1 kg of tissue was extracted in this way three times with one liter of solvent. The dry residue was dissolved in water, extracted three times with n-butanol and the combined butanol extracts were evaporated until dry.

The butanol extract of D. nipon, L. anthosticta and P. pectinifera was separated by chromatography in polychrom (Elyakov et al., 1976) into three fractions: inorganic substances and high-molecular proteins, eluted in the first batches of water; glycosides, eluted with water alcohol; and the lipid fraction, eluted with alcohol. The butanol extract of C. papposus and A. amurensis was diluted with hot methanol, which caused the proteins and inorganic matter to precipitate. At the same time, the methanol phase contained mainly lipids and glycosides.

After separation of the butanol extract, the aqueous residue contained mainly proteins. This extract was treated with acid and heated to denature

the proteins and obtain a set of amino acids and protein molecule radicals. In addition, an attempt was made to separate the aqueous part into two fractions differing in molecular mass of proteins, by dialysis. The fraction with m.m. $<$ 2000 which dialyzed through the membrane and the undialyzed residue with m.m. $>$ 2000 were evaporated dry.

All groups of substances were tested in solutions of identical concentration. A portion of extracts and glycosides was tested experimentally for the average noticeable reaction. The portion for extracts was 500 mg, for glycosides 50 mg per 300 mL of solution. In preparing working solutions of homogenates, extracts and substances, 20 mL of homogenate (substance) was used per 10 mL of working solution. The calculated concentration of substances in the experimental compartment was approximately 33 mg/L for homogenates, 1600 mg/L for cold extracts, 8 mg/L for extracts and 0.8 mg/L for glycosides.

Results

In all tests, A. amurensis and D. nipon asteroids showed only negative reaction to the proposed substances or there was no reaction (see Table).

Neither species reacted to living asteroids of the studied species. As a rule, there was no reaction to homogenates, extracts or substances from conspecific asteroids. The asteroids studied unambiguously avoided homogenates of liver and did not react to homogenates of the gonads of all the asteroid species studied. They did not react to cold water extracts of body walls of A. amurensis and D. nipon and avoided extracts of L. anthosticta and P. pectinifera. In most cases, the reaction to total extracts was the same as to aqueous extracts of the body of asteroids.

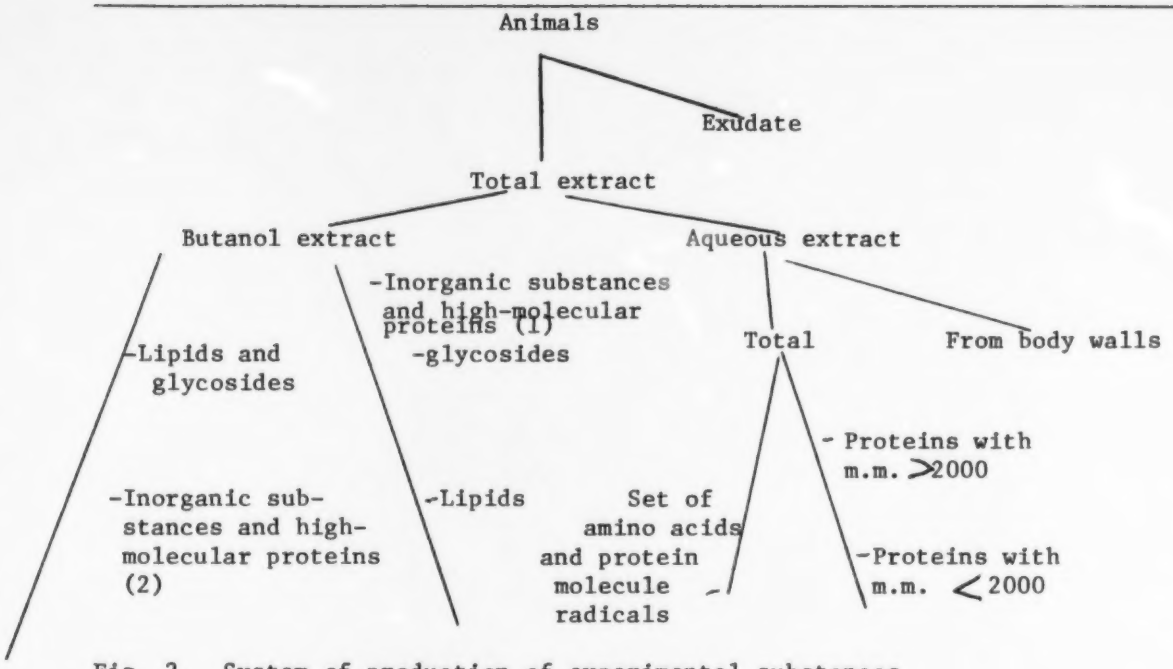

Fig. 2. System of production of experimental substances

Both species reacted to total water extracts and to water extracts of the body walls, even if there was no reaction to total extract of the given species. Denaturing of water extracts, as also milder denaturing, accompanying the production of exudates, leads to a breakdown /43 in the structure of native proteins. Asteroids did not react at all to either exudates or denatured water extracts.

After separation of the extract through the dialysis membrane, the experimental species continued to react to the protein fraction with m.m. > 2000 (except for the zero reaction of A. amurensis to D. nipon). We may thus consider that high-molecular proteins are one of the active sources of the reaction of asteroids to individuals of the same and different species.

The reaction to butanol extracts was on the whole more species-specific. In particular, there was no reaction to extracts from L. anthosticta

Total reaction of Distolasterias nipon (above the line) and Asterias amurensis (below the line) to starfish extracts and substances.

Суммарная реакция Distolasterias nipon (над чертой) и Asterias amurensis (под чертой) на экстракты и вещества, выделенные из звезд

Tissue, substance	Distolasterias nipon	Asterias amurensis	Patiria pectinifera	Lysastrosoma anthosticta	Crossaster papposus
Whole animal	0/0	0/0	0/0	0/0	—
Homogenate:	86/89	90/79	90/86	86/83	—
Liver	0/0	0/0	0/0	—	—
Gonads	0/0	0/0	79/82	82/100	—
Cold water extract of walls	0/0	0/0	0/0	0/0	—
Exudate	0/0	0/0	85/86	86/82	89/82
Extract:					
Total	0/0	0/0	0/0	73/79	90/86
Water	0/93	90/0	76/—	86/82	—
Water from walls	0/93	83/0	—	—	0/0
Water, denatured	0/0	0/0	0/0	0/0	0/0
Butanol	0/78	93/0	87/86	0/0	0/0
Proteins m.m. 2000	79/0	83/0	86/83	80/97	90/79
Proteins m.m. 2000	0/0	0/0	0/0	0/0	0/0
Lipids and glycosides	—	0/0	—	—	0/0
Lipids	0/0	0/0	0/0	—	0/0
Glycosides	0/0	0/0	0/0	0/0	—
Proteins and inorganic matter (1)	0/85	—	—	—	89/90
Proteins and inorganic matter (2)	—	—	—	—	79/79

Note. The figures are the percentage of negative reactions in the test total reliably differing from the percentage of negative reactions in the control.

and C. papposus. No reaction to glycosides and lipids at test concentrations was observed. The starfish reacted to a mixture of proteins and inorganic substances from the butanol extracts of some species. We may assume that

complexes of proteins and inorganic matter determined the reaction to the butanol extracts, and, together with high-molecular proteins, the total reactions of the asteroids.

The intensity of the reaction of test species varied considerably. /44 Thus, the reaction of A. amurensis to the protein fraction with m.m. $>$ 2000 from D. nipon and the reaction of D. nipon to the same fraction from A. amurensis was weaker than the reactions of these species to water extracts from the same asteroids. However, both species reacted more strongly to water extracts than to the protein high-molecular fraction from L. anthosticta and P. pectinifera.

It is noteworthy that both species reacted practically identically to every asteroid substance. By way of an exception, the reaction to the high-molecular protein fraction from C. papposus was considerably stronger in D. nipon than in A. amurensis.

Discussion

The pyloric ceca (hepatic sacs) of predatory asteroids contain enzymes of protease and amylase which are very similar in properties to tripsin of vertebrates (Camacho et al., 1970; Winter, Neurath, 1970; Bundy, Gustafson, 1973; Kozlovskaya, Elyakova, 1974; Elyakova, Kozlovskaya, 1975) and have a strong proteolytic action. The available data indicate the accumlation of digestive proteases in the pyloric ceca of asteroids in the form of inactive zymogen; there is an increase in activity with homogenization of tissues and other effects (Camacho et al., 1970; Bundy, Gustafson, 1973; Peng, Williams, 1973). It is apparently precisely these proteases which have been activated in homogenization that caused the very pronounced species-nonspecific escape reaction in the studied asteroid species.

It is reported in a number of works (Mauzey et al., 1968; Sloan, 1980) that asteroids excrete a certain "fright substance" when attacking their victims. This substance has a species-nonspecific action. These substances are also excreted by asteroids of the Solasteridae family, including Crossaster papposus, which attacks asteroids (Sloan, 1980; Van Veldhuizen, Oakes, 1981). In our experiments, the studied species reacted to substances isolated from all five starfish species, regarless of whether the species is a predator of the experimental species or not. The nature of this escape reaction had been noted previously - asteroids which were known not to be related in the predator-prey relationship nevertheless reacted to each other as potential enemies, exhibiting an active avoidance reaction (Mauzey et al., 1968; Hancock, 1974; Mayo, Mackie, 1976). It has been suggested (Sloan, 1979) that the fright substances excreted by asteroids do not determine interrelations of the predator-prey type, but rather competition for food.

The nature of the "fright substances" has not been adequately explained in existing studies. It is recognized that the substances that produce an avoidance reaction in molluscs are asterosaponins (Mackie et al., 1968; Mackie, Turner, 1970). In asteroids, the available data indicate that they are low-molecular factors of unknown nature associated: one - with a mixture of amino acids, the other - with nucleotides (Mayo, Mackie, 1976). The production of an escape reaction in Asterias amurensis and Distolasterias nipon, by the high-molecular protein fraction, as revealed in this paper, contradicts these data.

Our results can be explained by the physico-chemical properties of this fraction. The molecules of amino acids and nucleotides have a coefficient of diffusion in water on the order of 10^{-5} cm^2/s, while protein

macromolecules have a coefficient of 10^{-7} - 10^{-8} cm^2/s. If the water /45 is not mixed, the signal fields formed by the high-molecular proteins can exist for a considerably longer time than the fields formed by amino acids. This can be very significant for such slowly moving organisms as asteroids (Mackie, 1975). In fact, Heeb (1973) has shown that the high-molecular protein fraction of some molluscs attracts Asterias forbest.

This property of the high-molecular protein fraction (in pure form and in association with organic substances) of causing an avoidance reaction in asteroids is of interest for producing an "ecologically pure" repellent to control starfishes.

REFERENCES

1. Plokhinskii, N.A., 1970. Biometrics. Moscow. Moscow University, 367 pp.

Remaining references listed in English on attached copy of original.

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Литература

Плохинский Н. А. 1970. Биометрия. М.: МГУ, 367 с. **Bundy H. F., Gustafson J.** 1973. Purification and comparative biochemistry of protease from the starfish Pisaster giganteus. — Comp. Biochem. Physiol., v. 44B, N 1, p. 241—251. **Camacho Z., Brown J. R., Kitto G. B.** 1970. Purification and properties of trypsin-like proteases from the starfish Dermasterias imbricata. — J. Biol. Chem., v. 245, N 15, p. 3964—3972. **Castilla J. C., Crisp D. J.** 1973. Responses of Asterias rubens to water currents and their modification by certain environmental factors. — Neth. J. Sea Res., v. 7, p. 171—190. **Elyakov G. B., Levina E. V., Levin V. S., Kapustina I. I.** 1976. Glycosides of marine invertebrates. 5. A comparative study of glycoside fractions from starfish. — Comp. Biochem. Physiol., v. 55B, p. 57—60. **Elyakova L. A., Kozlovskaya E. P.** 1975. Proteinases of starfish. — Comp. Biochem. Physiol., v. 50B, N 2, p. 249—253. **Hancock D. A.** 1974. Some aspects of biology of the sunstar Crossaster papposus (L.). — Ophelia, v. 13, p. 1—30. **Heeb M. A.** 1973. Large molecules and the chemical control of feeding behavior in the starfish Asterias forbesi. — Helgoländ. Wiss. Meeresuntersuch., Bd. 24, S. 425—435. **Koziovskaya E. P., Elyakova L. A.** 1974. Purification and properties of trypsin-like enzymes from the starfish Lysastrosoma anthosticta. — Biochim. biophys. acta., v. 371, N 1, p. 63—70. **Mackie A. M.** 1975. Chemoreception. — In: Biochemical and biophysical perspectives in marine biology, v. 2. L.: Acad. Press, p. 69—105. **Mackie A. M., Lasker R., Grant P. T.** 1968. Avoidance reactions of a mollusc Buccinum undatum to a saponin-like surface active substances in the extracts of the starfish Asterias rubens and Marthasterias glacialis. — Comp. Biochem. Physiol., v. 26, p. 415—428. **Mackie A. M., Turner A. B.** 1970. Partial characterization of a biologically active steroid glycoside isolated from the starfish Marthasterias glacialis. — Biochem. J., v. 117, p. 543—550. **Mauzey K. P., Birkeland C., Dayton P. K.** 1968. Feeding behavior of asteroids and escape responses of their prey in the Puget Sound region. — Ecology, v. 49, p. 603—619. **Mayo P., Mackie A. M.** 1976. Studies of avoidance reactions in several species of predatory British seastars (Echinodermata: Asteroidea). — Mar. Biol., v. 38, p. 41—49. **Peng R. K. Y., Williams D. C.** 1973. Partial purification and some enzymatic properties of proteolytic enzyme fractions isolated from Pisaster ochraceus (Echinoderm.) pyloric caeca. — Comp. Biochem. Physiol., v. 44B, N 4, p. 1207—1217. **Pratt D. M.** 1974. Attraction to prey and stimulus to attack in the predatory gastropod Urosalpinx cinerea. — Mar. Biol., v. 27, p. 37—45. **Sloan N. A.** 1979. Starfish encounters: an experimental study of its advantages. — Experientia, v. 35, p. 1314—1315. **Sloan N. A.** 1980. Aspects of the feeding biology of asteroids. — Oceanogr. Mar. Biol. Annu. Rev., v. 18, p. 57—124. **Sloan N. A., Northway S. M.** 1982. Chemoreception by the asteroid Crossaster papposus (L.). — J. Exp. Mar. Biol Ecol., v. 61, p. 85—98. **Van Veldhuizen H. D., Oakes V. J.** 1981. Behavioral responses of seven species of asteroids to the predator, Solaster dawsoni (Responses of asteroids to the predator Solaster dawsoni). — Oecologia (Berl.), v. 48, N 2, p. 214—220. **Winter W. B., Neurath H.** 1970. Purification and properties of a trypsin-like enzyme from the starfish Evasterias troschelli. — Biochemistry, v. 9, N 24, p. 4673—4679.

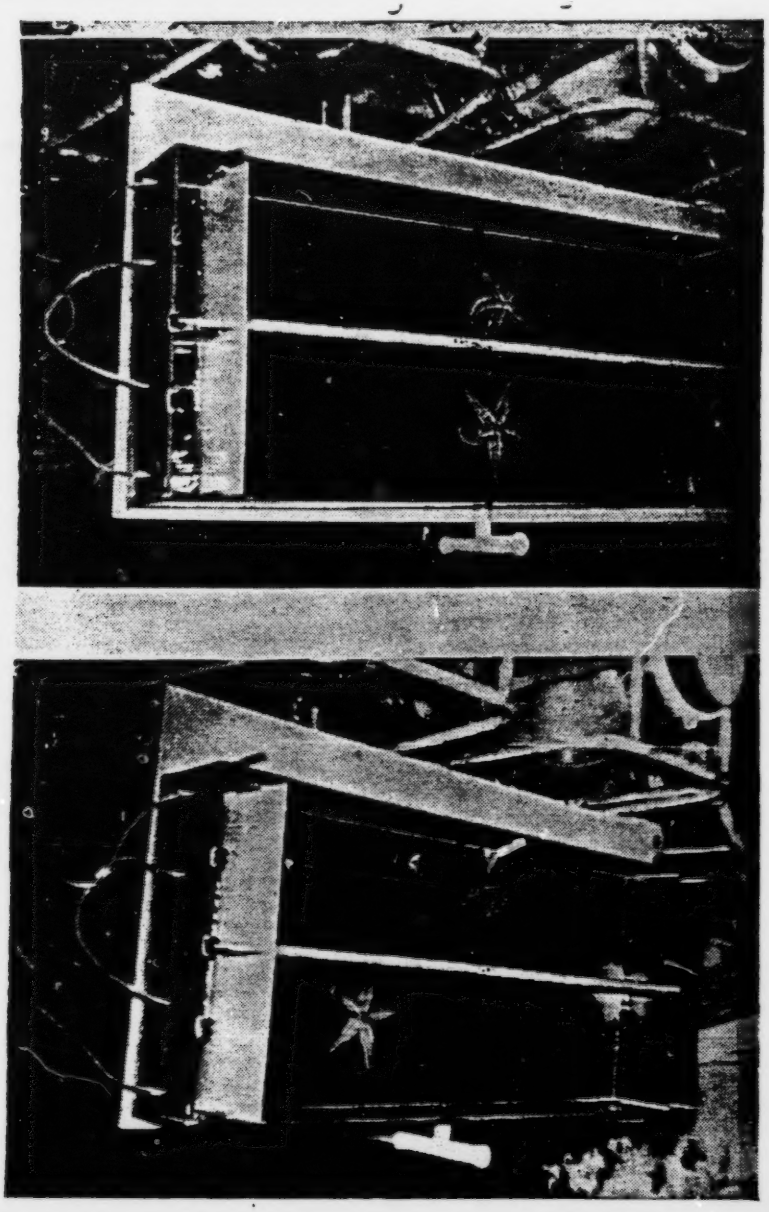

Fig. 1. General appearance of the experimental device. Two phases of the experiment.